

Increased frequency of the null allele at the complement C4b locus in autism

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SUMMARY

Associations between C4 deficiency and autoimmune disorders have been found over the past several years. Since autism has several autoimmune features, the frequencies of null (no protein produced) alleles at the C4A and C4B loci were studied in 19 subjects with autism and their family members. The autistic subjects and their mothers had significantly increased phenotypic frequencies of the C4B null allele (58% in both the autistic subjects and mothers, compared with 27% in control subjects). The siblings of the autistic subjects also had an increased frequency of the C4B null allele, but this increase was not significant. The fathers had normal frequencies of this null allele. All family members had normal frequencies of the C4A null allele, all normal C4A and C4B alleles and all BF and C2 alleles.

Keywords autism complement C4B null alleles

INTRODUCTION

Autism is a severe developmental disorder characterized by abnormalities in the manner in which the brain collects and integrates information resulting in abnormal communication and social skills. The cause of autism remains unknown. However, recent investigations suggest that this disorder shares several features of established autoimmune disorders, including genetic susceptibility (Folstein & Rutter, 1977; Smalley, Asarnow & Spence, 1988), helper T cell alterations (Stubbs *et al.*, 1977; Warren *et al.*, 1986, 1990; Yonk *et al.*, 1990) and other immune abnormalities (Weizman *et al.*, 1982; Todd & Ciarello, 1985), association with congenital viral infections (Chess, 1977; Stubbs, 1978); and a four-to-five times greater occurrence in boys than in girls.

Recent evidence suggests that inherited abnormalities of the complement C4 proteins may be linked to certain autoimmune diseases (reviewed by Kahl & Atkinson, 1988). C4 is composed of two distinct but highly homologous glycoproteins, C4A and C4B, whose structural genes are found within the MHC on chromosome 6. C4A and C4B genes are highly polymorphic with each having a null (Q0) allele which is functionally silent (no protein is produced). Homozygosity for the null allele at C4A or C4B, which is quite rare, is associated with slightly reduced C4 serum levels. Heterozygotes have a null allele on one of their chromosomes (at either C4A or C4B) at a frequency of

approximately 36% and 30%, respectively, in the general population and C4 protein levels which are usually in the lower end of the normal range (reviewed by Kahl & Atkinson, 1988).

The frequency of C4-deficiency (null alleles at the C4A locus) is increased among patients with SLE (Fielder *et al.*, 1983; Christiansen *et al.*, 1983), insulin-dependent diabetes mellitus (Raum, Awdeh & Alper, 1981) and scleroderma (Briggs *et al.*, 1986). An increased frequency of null alleles at the C4B locus has been reported in patients with scleroderma (Mollenhauer *et al.*, 1984) and schizophrenia (Rudduck *et al.*, 1985). Since autism displays several characteristics of autoimmunity, we have performed analysis of extended haplotypes in autism.

SUBJECTS AND METHODS

Included in this investigation were family members of 190 randomly chosen autistic subjects (17 male and two female), all of Northern European descent. Each family included one autistic child and both parents. In 14 of these families, one normal sibling without the symptoms of autism was also available to be studied. The diagnosis of autism satisfied DSM-III-R criteria for infantile autism as ascertained by at least two psychiatrists or psychologists. None of the autistic subjects had an identifiable cause of their disease and all were living at home at the time of study. Also in the investigation were 62 randomly chosen normal subjects also of Northern European descent, unrelated to the autistic subjects, who were living in the same geographical area (Northern Utah) as the autistic subjects.

Serum samples were obtained from the subjects following informed consent procedures. The genetic typing for the

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complement proteins was performed by The Center for Blood Research Laboratories, Boston, MA, under the direction of Dr David H. Bing, using previously described techniques (Marcus & Alper, 1986). Serum samples were incubated with neuraminidase from *Clostridium perfringens* overnight at room temperature with continuous dialysis against 0.1 M phosphate buffer, pH 7.0, containing 0.005 M EDTA-Na₂. The desialated samples were subjected to electrophoresis and immunofixation with goat anti-human C4 (Atlantic Antibodies, Scarborough, ME) at 1% and subjected to crossed immunoelectrophoresis. Some samples, processed as above, were developed with a C4 complement overlay consisting of antibody-sensitized sheep erythrocytes and C4- deficient guinea pig serum incorporated into a gel and layered onto a C4 agarose gel. The presence of null alleles (QO) was determined by inspection of immunofixation patterns or by crossed immunoelectrophoresis. The detection of null alleles in heterozygotes requires the quantification of all gene products and analyses of their ratios. This allowed identification of genuine null alleles and reduced the likelihood of an incorrect assignment of a null allele that may have resulted from an ambiguous situation such as the expression of the same C4 allotype at both C4 loci.

BF typing was carried out with frozen plasma that was subjected to electrophoresis in agarose gel and immunofixation with goat antiserum to human factor B (Atlantic Antibodies) as previously described. Typing for the C2 complement proteins was performed by isoelectric focusing of the samples in polyacrylamide gel and an overlay agarose gel containing antibody-sensitized sheep erythrocytes and diluted fresh normal human serum.

Data were analysed by χ^2 analysis with 2 x 2 contingency tables.

RESULTS

Genotypes of the complement alleles BF, C2, C4A and C4B in autistic subjects and family members are given in Table 1. A summary of the C4A and C4B typing is presented in Table 2. Overall, the gene frequency of the C4A null allele was not significantly changed in any of the autistic subjects or any of their family members, compared with the normal controls. However, the gene frequency of the C4B null allele was significantly increased in the autistic subjects ($P=0.03$, by χ^2 contingency table analysis). Moreover, the mothers of the autistic subjects had a gene frequency of C4B that was also elevated ($P=0.01$). On a phenotypic basis, 11 out of the 19 (58%) autistic subjects and 11 out of the 19 mothers had the C4B null allele on one of their chromosomes as compared with that of 17 out of 64 (27%) of the unrelated normal subjects ($P=0.14$ in both cases). The siblings had a frequency of the C4B null allele which was increased (Table 2), but not significantly; the paternal frequency for C4B was not elevated.

The frequency of BF and C2 alleles are given in Table 3. No alteration in frequencies with any of these alleles was found.

DISCUSSION

This study provides preliminary evidence for a temporal link between the C4B null allele and autism. Statistically significant findings were obtained, although the study included only a limited number of subjects. Moreover, comparison of the

Table 1. Complement alleles in family members of autistic subjects

Subject no.	Chromosomes from mothers BF-C2-C4A-C4B*		Chromosomes from fathers BF-C2-C4A-C4B*	
	No. 1 (autistic)†	No. 2 (other)	No. 1 (autistic)†	No. 2 (other)
1	S-C-3-QO	<i>S-C-6-1</i>	<i>S-C-3-1</i>	S-C-3-1
2	S-C-2-(2,1)‡	<i>S-C-3-QO</i>	<i>S-C-3-1</i>	S-C-QO-1
3	<i>S-C-3-QO</i>	S-C-QO-1	<i>S-C-QO-1</i>	S-C-3-1
4	S-C-QO-3	S-C-3-QO	S-C-3-2	S-C-QO-1
5	<i>S-C-3-QO</i>	F1C-QO-1	<i>S-C-QO-1</i>	S-C-3-1
6	S-C-3-1	S-C-3-1	S-C-3-1	S-C-QO-3
7	S-C-3-QO	S-C-3-1	S-C-QO-1	F-C-3-1
8	<i>S-C-QO-1</i>	S-C-3-1	<i>S-C-3-2</i>	S-C-QO-1
9	<i>S-C-6-1</i>	S-C-QO-2	S-C-3-QO	<i>S-C-QO-1</i>
10	F-C-3-1	<i>S-C-3-1</i>	S-C-3-1	<i>S-C-3-QO</i>
11	F-C-3-1	<i>S-C-QO-2</i>	S-C-3-QO	<i>F-C-3-1</i>
12	<i>S-C-QO-1</i>	S-C-6-1	F-C-3-QO	<i>F-C-3-1</i>
13	F-C-3-1	S-C-3-QO	F-C-(3,2)-QO§	F-C-3-1
14	<i>S-C-6-1</i>	S-C-3-QO	S-C-3-1	<i>F-C-2-QO</i>
15	S-C-QO-1	<i>F-C-3-1</i>	S-C-3-1	<i>S-C-3-1</i>
16	<i>S-C-3-QO</i>	F-C-3-1	F-C-3-1	<i>S-C-QO-1</i>
17	S-C-3-1	<i>S-?-4-2</i>	S-C-6-1	<i>F-C-3-1</i>
18	S-C-3-QO	<i>S-C-3-QO</i>	<i>S-?-4-5</i>	S-C-3-1
19	S-C-3-QO	S-C-3-1	S-C-3-1	S-C-3-1

Alleles received by normal siblings are italicized.

* Complement alleles given in order of BF-C2-C4A-C4B.

† Alleles segregating to autistic children presented as maternal and paternal chromosomes number 1.

‡ Expressed the relatively common duplicated C4B allele (2,1).

§ Expressed the duplicated C4A allele (3,2).

Table 2. C4 Complement types in families of autistic subjects

C4 Type	Number with complement type and gene frequency				
	Autistic (n=38)	Sibling (n=28)	Maternal (n=38)	Paternal (n=38)	Normal (n=124)
C4A					
1	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.01)
2	2 (0.05)	1 (0.04)	1 (0.03)	3 (0.08)	4 (0.03)
3	26 (0.68)	15 (0.54)	24 (0.63)	26 (0.68)	74 (0.60)
4	1 (0.03)	2 (0.07)	1 (0.03)	1 (0.03)	17 (0.14)
6	3 (0.08)	3 (0.11)	4 (0.11)	1 (0.03)	3 (0.02)
Q0	7 (0.18)	7 (0.25)	8 (0.21)	8 (0.21)	25 (0.20)
C4B					
1	23 (0.61)	17 (0.61)	22 (0.58)	28 (0.74)	89 (0.72)
2	3 (0.08)	3 (0.11)	4 (0.11)	2 (0.05)	15 (0.12)
3	1 (0.03)	0 (0.00)	1 (0.03)	1 (0.03)	1 (0.01)
5	1 (0.03)	1 (0.04)	0 (0.00)	1 (0.03)	2 (0.02)
Q0	11(0.29)*	7 (0.25)	12 (0.32)†	6 (0.16)	17 (0.14)

* $P=0.03$.

† $P=0.01$.

Table 3. BF and C2 alleles in families of autistic subjects

Component	Number with allele and gene frequency				
	Autistic (n = 38)	Sibling (n = 28)	Maternal (n = 38)	Paternal (n = 38)	Normal (n = 124)
BF					
S	32 (0.84)	23 (0.82)	32 (0.84)	29 (0.76)	101 (0.81)
F	6 (0.16)	5 (0.18)	5 (0.13)	9 (0.24)	18 (0.15)
F1	0 (0.00)	0 (0.00)	1 (0.03)	0 (0.00)	3 (0.02)
S1	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.02)
C2*					
C	37 (0.97)	26 (0.93)	37 (0.97)	37 (0.97)	116 (0.94)
B	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.01)

*C2 typing on chromosomes of one autistic subject, two siblings, one mother, one father and seven normal subjects was uninterpretable.

frequency of the C4B null frequency in our normal unrelated subjects (27%) with that reported in the literature (30%) (reviewed by Kahl & Atkinson, 1988) provides additional support for a link between the C4B null allele and autism. However, these findings must be interpreted with caution because 11 out of the mothers as well as four out of the siblings, three of whom were younger than the autistic subjects, all without the symptoms of autism, expressed the C4B null allele. Therefore, aetiological agents, in addition to the possible involvement of the C4B null allele, must be responsible for the development of autism. For example, as reviewed above, viral infections and immune abnormalities also have been associated with autism. Thus, it is possible that a combination of a C4B null allele, a virus, an immune element and/or other agents interact in causing this severe developmental disorder.

Due to preliminary nature of this report, it would probably be premature to present an extensive discussion on the possible mechanism by which partial deficiency of C4 complement proteins predisposes to autism. However, one could speculate that complement deficiency allows an infectious agent or immune complex to persist, resulting in prolonged immunological stimulus and triggering of an autoimmune reaction. The fact that the mothers of the autistic children expressed the C4B null allele at the same frequency as their autistic children might imply that a partial maternal complement deficiency also contributes to the development of some cases of autism. Perhaps, during the time that the mother was carrying the autistic child, she did not clear a viral infection in immunocompetent fashion, resulting in an intrauterine fetal infection that damaged the developing central nervous system or triggered an autoimmune response. Circumstantial evidence for this possibility are reports of association of congenital infections of rubella (Chess, 1977) and cytomegalovirus (Stubbs, 1978) with autism.

Another possible explanation for an association of the C4B null allele with autism is by virtue of the location of the structural gene for this allele within the major histocompatibility complex on chromosome 6. The null allele of C4B has been reported to be strong linkage disequilibrium with HLA-DR4 and HLA-B44 (Awdeh *et al.*, 1983). Perhaps one of these alleles or some other gene linked to C4B is related to the development of autism. Our preliminary findings suggest that a thorough investigation is needed into major histocompatibility markers in autism.

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